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ADENOSINE PRETREATMENT FOR PROLONGED CARDIAC STORAGE

An evaluation with St. Thomas' Hospital and University of Wisconsin solutions

Adenosine pretreatment has been shown to be beneficial in several models of ischemia-reperfusion. We wished to evaluate whether adenosine pretreatment is cardioprotective for prolonged cardiac storage and whether the presence of adenosine in the storage media affects the results. Isolated rodent hearts were obtained from Sprague-Dawley rats, mounted on a Langendorff apparatus, instrumented with an intraventricular balloon, and ventricularly paced at 300 beats/min. Four groups of hearts were studied in a 2 × 2 factorial experiment (n = 8 to 12 per group). Hearts were subjected to normal perfusion or to solution supplemented with adenosine 50 μmol/L for 10 minutes followed by adenosine-free perfusion for 10 minutes. Hearts then were stored for 8 hours at 0° C in either University of Wisconsin solution (adenosine 5 mmol/L) or St. Thomas' Hospital II solution (adenosine free). Adenosine pretreatment increased tissue levels of adenosine triphosphate before storage (p = 0.04). Nonfunction was less common after storage (1/19 versus 6/20 hearts, $p < 0.05$), and diastolic function was better preserved in the adenosine groups in the reperfusion phase (p = 0.01). The beneficial effects of adenosine pretreatment were independent of which storage solution was used. Developed pressure was increased ($p < 0.05$) and release of creatine kinase and lactate dehydrogenase was reduced ($p < 0.0001$) in hearts treated with University of Wisconsin solution compared with those treated with St. Thomas' Hospital solution.

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These studies suggest that adenosine pretreatment improves recovery after prolonged hypothermic storage and that the presence of adenosine in the preservation solution does not alter the results. The experiments provide further evidence that extended myocardial protection is better enhanced with University of Wisconsin solution than with St. Thomas' Hospital II solution. (J THORAC CARDIOVASC SURG 1995;110:293-301)

The cardioprotective properties of adenosine have been well established.[1] With respect to cardiac transplantation, adenosine supplementation has been shown to be beneficial for extended perfusion,[2] when added to University of Wisconsin solution (UWS) for prolonged hypothermic storage,[3] and in the reperfusion phase after prolonged storage with St. Thomas' Hospital cardioplegic solution.[4] Masuda and associates[5] determined that the nucleoside transport blocker R75231 did enhance cardiac recovery. Therefore additional studies regarding adenosine metabolism and prolonged hypothermic storage appeared to be justified. In the following series of experiments, we have determined whether adenosine pretreatment improves poststorage results in hearts protected with either UWS (adenosine 5 mmol/L) or the adenosine-free solution, St. Thomas' Hospital II solution (STS).

Methods

Hearts were obtained from Sprague-Dawley rats (250 to 500 gm). All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital. Heparin 200 units was administered intravenously. A median sternotomy was performed and the hearts were rapidly excised and immersed in chilled normal saline solution. After excision, hearts were perfused on a Langendorff apparatus with filtered Krebs-Henseleit buffer (composition in millimoles per liter: NaCl 118, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5 $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11) with a pressure of 100 cm H_2O. The elapsed time from sternotomy to buffer perfusion was approximately 45 seconds. The hearts were ventricularly paced at 300 beats/min. The reservoirs and conduits were wrapped in a water jacket at 37° C. The perfusate was gassed with 95% oxygen and 5% carbon dioxide, and the pH was adjusted to 7.4. A saline-filled balloon was inserted in the left ventricle via a left atriotomy and fixed to the mitral valve ring with a purse-string suture. The balloon volume was varied in 0.02 ml increments from 0 to 0.4 ml not to exceed an end-diastolic pressure of 30 mm Hg. Data were obtained after a 30-minute stabilization period before storage and after 45 minutes of reperfusion after storage.

Developed pressure was recorded before and after storage with a preischemic balloon volume associated with an end-diastolic pressure of 5 mm Hg. Hearts were rejected for subsequent storage if the developed pressure was less than 80 mm Hg. Developed pressure after storage was not obtained if the end-diastolic pressure exceeded 30 mm Hg with the 5 mm Hg preischemic balloon volume. Compliance curves were assessed by linear regression analysis of the end-diastolic pressure-volume data to calculate a slope and an X intercept. We[6] have previously demonstrated that linear regression provides a reasonable model for diastolic function curves. Coronary flow was obtained in duplicate by timed collection in the empty beating state.

Cardiac tissue levels of adenine nucleotides, nucleosides, and degradation products were determined by high-performance liquid chromatography as previously described.[7, 8] Results are expressed as micromoles per gram dry weight.

Creatine kinase release and lactate dehydrogenase release were assessed during the 45 minutes of reperfusion after the storage interval. The entire coronary effluent was collected. Enzyme release was evaluated with a Hitachi Automatic Analyzer 737 (Hitachi Ltd., Tokyo, Japan) and an Olympus AU 800 Analyser (Olympus Corp., Lake Success, N.Y.), respectively, using spectrophotometry at 340 nm. The results of these studies are recorded as international units per gram dry cardiac weight.

Study protocol. Adenosine was obtained from Sigma Chemical Co. (St. Louis, Mo.). Hearts were divided into four groups. A 2 × 2 factorial study design was used to evaluate the main effects of adenosine pretreatment versus control perfusion and UWS versus STS (n = 8 to 12 per group). Functional data were obtained after 30 minutes of Langendorff perfusion before ischemia. Hearts underwent either an additional 20 minutes of unmodified perfusion or adenosine pretreatment (10 minutes of perfusion supplemented with a 50 μmol/L dose of adenosine followed by 10 minutes of adenosine-free perfusion). Hearts then underwent aortic root flushing (15 ml/kg) and storage (15 to 20 ml) in UWS or STS for 8 hours at 0° C.

In parallel experiments for the assessment of tissue levels of purine metabolites, hearts were mounted on the Langendorff apparatus, equilibrated for 30 minutes, and subjected to the adenosine pretreatment protocol or unmodified perfusion (n = 6 per group). Hearts were then immediately immersed in liquid nitrogen for subsequent analysis (i.e., prestorage biopsy tissue).

Statistical analysis. Data analysis was facilitated by means of the Statistical Analysis System software (SAS

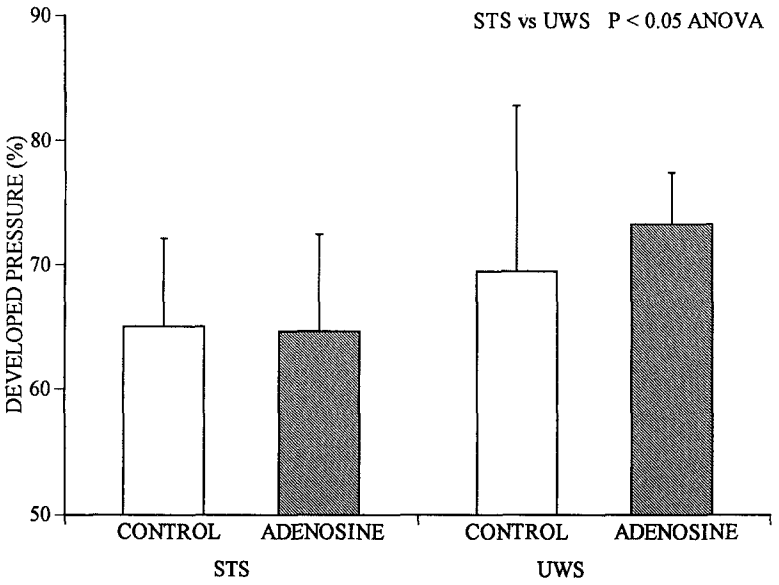

Fig. 1. Developed pressure as a percentage of prestorage values (mean ± standard deviation, $n = 7$ to 11 per group) are presented for each of the four treatment groups. There was no difference between groups for adenosine perfusion versus control, but there was better recovery of poststorage developed pressure in the UWS-treated groups. *ANOVA,* Analysis of variance.

Institute, Cary, N.C.) and a microcomputer. Categoric variables are expressed as an absolute frequency. Continuous variables are expressed as a mean ± standard deviation of the original values, as a percentage of control, or as a percentage reduction. Data analysis for categoric variables was performed by a χ^2 test. Data analysis for continuous variables was performed with a two-way analysis of variance by evaluating the main effects of adenosine pretreatment, storage solution, and their interactions.[9] Diastolic function was assessed with repeated-measures analysis of variance for evaluation of slope and X intercept.[9] Statistical significance is assumed for $p < 0.05$.

Results

Adenosine versus control. Before storage, developed pressure decreased during adenosine administration to 87.8% ± 6.7% ($p < 0.0001$) of preadministration values and returned to 93.7% ± 5.2% before storage ($p < 0.0001$). Coronary flow increased to 115.7% ± 15.4% ($p < 0.0001$) of preadenosine values during infusion and decreased to 104.9% ± 5.5% ($p < 0.0001$) before cardiac storage. This corresponds to a delivered adenosine dose of 12.0 ± 1.4 μmol per animal. Tissue levels of adenosine triphosphate (ATP) were increased whereas levels of adenosine diphosphate and monophosphate were decreased in the adenosine pretreatment groups immediately before storage (Table I).

Altogether there were seven hearts (1/19 adenosine pretreatment versus 6/20 buffer perfusion) in which developed pressure could not be recorded after storage (because the poststorage, end-diastolic pressure exceeded the threshold value of 30 mm Hg at the preischemic balloon volume associated with an end-diastolic pressure of 5 mm Hg). The distribution of hearts exhibiting nonfunction were one heart treated with STS and adenosine versus three STS control and three UWS control hearts ($p < 0.05$). No other differences with respect to developed pressure were measurable between control and adenosine-pretreated hearts (Fig. 1). Diastolic

Table I. *Prestorage cardiac tissue metabolites*

	Control ($n = 6$)	*Adenosine* ($n = 6$)	*p Value*
ATP (μmol/gm)	10.9 ± 1.4	13.0 ± 1.5	0.04
ADP (μmol/gm)	7.2 ± 1.2	4.9 ± 1.4	0.02
AMP (μmol/gm)	1.6 ± 0.6	0.6 ± 0.7	0.04
TAN (μmol/gm)	19.7 ± 1.1	18.5 ± 1.0	0.10
Adenosine (μmol/gm)	0.13 ± 0.01	0.09 ± 0.03	0.04
Inosine (μmol/gm)	0.35 ± 0.02	0.33 ± 0.03	0.35
Hypoxanthine (μmol/gm)	0.039 ± 0.02	0.038 ± 0.011	0.91
Xanthine (μmol/gm)	0.030 ± 0.005	0.021 ± 0.003	0.008

The acute effects of adenosine pretreatment on purine metabolites measured 10 minutes after adenosine infusion immediately before storage. Values represent mean ± standard deviation. *ATP,* Adenosine triphosphate; *ADP,* adenosine diphosphate; *AMP,* adenosine monophosphate; *TAN,* total adenine nucleotides; Σ (ATP + ADP + AMP).

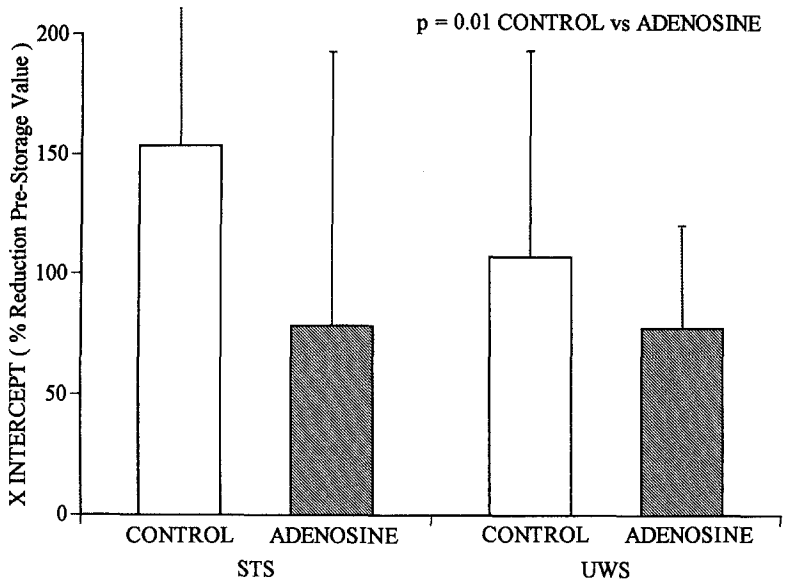

Fig. 2. The percent reduction of the X intercept from before to after storage (mean ± standard deviation, $n = 8$ to 12 per group) derived from the diastolic function analysis is presented. There was a large reduction of the X intercept for all groups (shift to the *left*), which was limited in the adenosine-treated hearts. There was no significant difference between STS and UWS groups. *ANOVA,* Analysis of variance.

Table II. *Diastolic function*

		Slope (mm Hg/ml)		*X intercept (ml)*	
Group	*N*	*Before storage*	*After storage*	*Before storage*	*After storage*
STS control	10	87.9 ± 18.3	175.0 ± 167.2	0.093 ± 0.034	−0.041 +0.052
STS and adenosine	12	96.4 ± 24.0	133.8 ± 44.4	0.078 ± 0.037	−0.010 +0.038
UWS control	10	113.7 ± 30.9	137.1 ± 88.6	0.116 ± 0.047	0.011 ± 0.069
UWS and adenosine	8	94.0 ± 18.2	117.8 ± 14.1	0.090 ± 0.029	0.020 ± 0.037
p Value (ANOVA)		Storage	0.0067	Storage	<0.0001
p Value (ANOVA)		Storage * adenosine	0.4145	Storage * adenosine	0.0118
p Value (ANOVA)		Storage * solution	0.2035	Storage * solution	0.1384
p Value (ANOVA)		Storage * adenosine * solution	0.4060	Storage * adenosine * solution	0.7143

There was a significant increase in slope and shift to the left of the X intercept after storage. The reduction of X intercept was decreased in the adenosine-treated hearts (storage * adenosine effect). The *asterisks* refer to the interaction term.

function results for slope and X intercept are presented in Table II. Overall, a large increase in slope and a decrease in X intercept were observed from prestorage to poststorage values. The increase in slope was not influenced by the presence of adenosine, although the reduction in X intercept was limited in the adenosine-treated hearts ($p = 0.0118$). The poststorage X intercept, expressed as a percent reduction of prestorage values, is presented in Fig. 2. No differences in coronary flow were noted among any of the groups, with values ranging from 60.3% ± 14.9% to 64.6% ± 10.2%. No differences in creatine kinase (Fig. 3) or lactate dehydrogenase release (Fig. 4) were noted between the control and adenosine-treated hearts.

STS versus UWS. The return of developed pressure was greater after storage in the UWS versus the STS groups (Fig. 1). No significant differences in the poststorage diastolic function were observed, although the decrease in X intercept tended to be less for both UWS groups ($p = 0.1384$). The decreases in creatine kinase release (Fig. 3) and lactate dehydro-

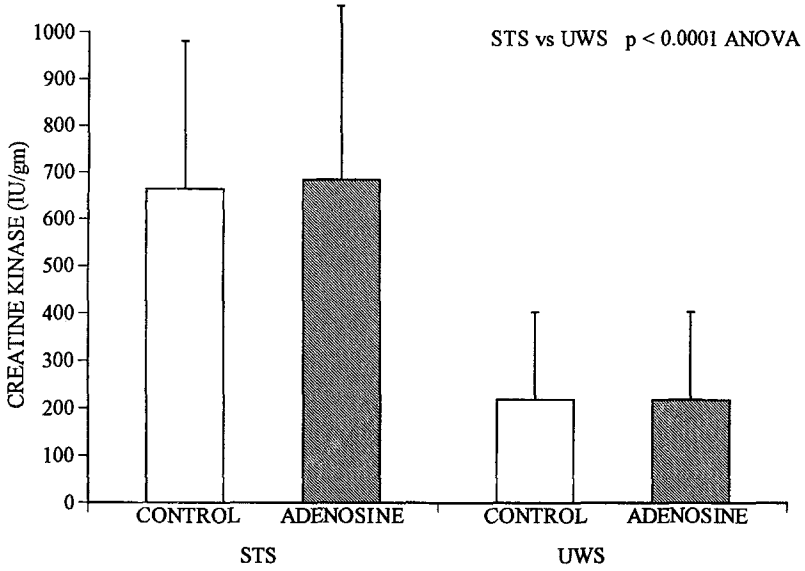

Fig. 3. Results for creatine kinase release during the 45-minute reperfusion phase are presented. The values for the UWS groups were significantly lower than those for the STS hearts. There was no significant difference between control and adenosine pretreatment. *ANOVA,* Analysis of variance.

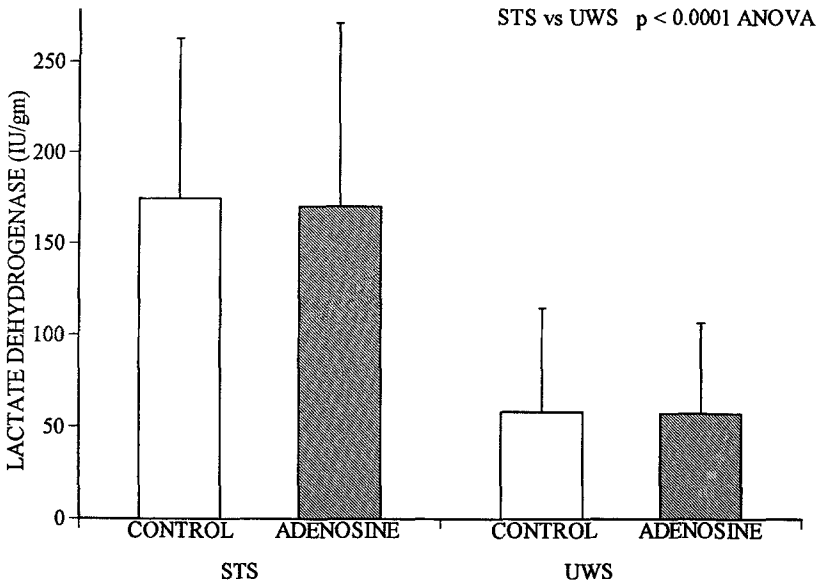

Fig. 4. The results for lactate dehydrogenase (mean ± standard deviation, $n = 8$ to 12 per group) are presented. There was a significant reduction in the UWS-treated hearts compared with STS groups. There was no reduction noted for adenosine pretreatment. *ANOVA,* Analysis of variance.

genase release (Fig. 4) were highly significant ($p < 0.0001$) in the UWS groups compared with the STS groups.

Discussion

Our study was designed to assess the potential benefit of adenosine pretreatment before prolonged hypothermic storage, similar to the documented improvement noted with models of normothermic global ischemia.[10-12] Important differences were identified with adenosine pretreatment with respect to prestorage adenine nucleotides, diastolic dysfunction, and, as a result of excessive end-diastolic pressures, measurable systolic function. These

changes in left ventricular diastolic parameters with adenosine would be extremely helpful after cardiac transplantation (especially in recipients with preexisting pulmonary hypertension) with an allograft right ventricle unaccustomed to the increased afterload. The changes noted in our experiments occurred irrespective of which storage solution was used for the studies.

The cardioprotective role of adenosine has been evaluated in many ischemia-reperfusion models including global ischemia,[10-12] regional ischemia,[13, 14] cardioplegia,[15, 16] and ventricular assist.[17] Murray and colleagues[18] initially described ischemic preconditioning whereby brief episodes of coronary ischemia protect against subsequent prolonged coronary occlusions. The mechanisms responsible for ischemic preconditioning are uncertain, although reports by Liu,[19] Thornton,[20] and their colleagues have provided evidence to implicate adenosine A_1-selective receptor activation as an important event (caused by interstitial adenosine accumulation). Adenosine or A_1-selective agonists mimic the protection by ischemic preconditioning, whereas A_2-selective agonists are ineffective and the temporal pattern is consistent with preconditioning. Adenosine receptor antagonists block the cardioprotection provided by ischemic preconditioning. Although the data supporting adenosine are persuasive, the ultimate effector is incompletely understood.

Adenosine supplementation of extended cardiac allograft preservation helped maintain left ventricular blood flow.[2] Adenosine was included in the UWS formulation because ATP synthesis was facilitated during hypothermic kidney perfusion.[21] More recent studies by Lasley and Mentzer[3] directly evaluated the contribution of adenosine to UWS for cardiac preservation. Interstitial adenosine concentrations were 20 to 40 times greater according to microdialysis and improvement in functional recovery. Other authors have examined the role of either a nucleoside transport blocker[5] or acadesine[22] and documented improvement after hypothermic storage. It appears that maneuvers to enhance endogenous adenosine or provision of exogenous adenosine exert positive effects for extended cardiac allograft preservation, similar to those achieved in other ischemia-reperfusion models.

We previously conducted experiments evaluating the addition of a nucleoside transport blocker[23] to UWS and determined that additional manipulations of adenosine metabolism further enhance cardiac recovery. We were therefore stimulated to explore whether adenosine pretreatment would be effective. Because we were uncertain whether the adenosine content of UWS would obscure any putative adenosine pretreatment contribution, a factorial study design was used in which both pretreatment and study solution were analyzed simultaneously. Accordingly, differences resulting from the effects of adenosine administration versus storage conditions have been separately reported. The adenosine protocol used in these studies was selected after pilot experiments ($n = 2$ per group) evaluating adenosine concentrations of 25 to 100 μmol/L with and without an adenosine-free interval after the adenosine infusion.

It cannot be ascertained whether the improvement noted in the current experiments was mediated by A_1-receptor or A_2-receptor activation (or both) or was not receptor mediated. During the adenosine infusion, developed pressure was reduced, which suggests A_1-receptor activation, whereas coronary flow increased, which is indicative of A_2-receptor activation. Bradycardia occurred during the adenosine infusion in our pilot studies, likely related to A_1-receptor activation (which was prevented during our reported experiments by pacing all hearts). For the preconditioning effect, the evidence from the literature is most supportive of A_1-receptor involvement. Additional investigations involving selective A_1-receptor or A_2-receptor agonists would help determine which receptor is primarily affected. Furthermore, we recognize that the buffer-perfused, Langendorff apparatus has significant limitations and that experiments involving blood perfusion would be helpful.

Our biopsy results differentiated between control perfusion and adenosine pretreatment with respect to adenine nucleotides and nucleosides. Our protocol indicates an important non-receptor-mediated contribution of adenosine. This contribution may simulate the clinical situation, in which hearts from organ donors may be ischemically damaged before harvest. It is conceivable that adenosine may facilitate de novo adenine nucleotide synthesis in such circumstances. Cardiac biopsies were performed 10 minutes after adenosine infusion, at which time adenosine and xanthine levels were reduced, whereas inosine and hypoxanthine were similar in the adenosine pretreated and the control hearts. It is probable that biopsy tissue obtained during adenosine infusion would have demonstrated increased nucleosides and degradation products.

Ischemic contracture has been proposed as one of

the key limitations for extended cardiac storage[24] and may explain the differential temporal protection for abdominal versus cardiac allografts provided by UWS. The increase in diastolic pressure was tightly correlated with the decrease in tissue content of ATP in rabbit studies reported by Stringham and associates.[24] The beneficial response that we detected for diastolic parameters with adenosine pretreatment may relate to the greater ATP content before storage. Other studies suggest that adenosine pretreatment slows the decline of ATP and limits intracellular calcium accumulation,[25] both of which would favorably affect contracture development.

One of the potential benefits of adenosine is vascular dilatation. Dilatation is pertinent because cardioplegic solutions[26] and UWS[27, 28] appear to impair coronary endothelium-dependent relaxation, which may be mediated in part by oxygen-derived free radical injury.[29] Conflicting evidence has been reported concerning the role of adenosine in limiting microcirculatory dysfunction,[30, 31] which may be due to model differences. Galiñanes and Hearse[4] have reported that adenosine administered during reperfusion hastened early recovery of contractile function and coronary flow of transplanted hearts. In any case, global coronary flow was reduced equally after storage in each of the four groups in our experiments, and no apparent improvement with adenosine pretreatment was detected.

A valid concern regarding the results of these experiments is the applicability of adenosine infusion into clinical practice. Inotrope-dependent donors may be intolerant of large-dose adenosine infusion caused by hypotension. Adenosine administration could require cardiopulmonary support or could be performed during ex vivo perfusion immediately after cardiac excision. Alternatively, A_1-receptor agonists rather than adenosine may be better tolerated in such circumstances.

The second principal result of these experiments is that UWS is preferred instead of STS for extended myocardial preservation. With notable exceptions,[27, 32] experimental hypothermic cardiac storage with UWS is better than storage with other storage media.[3, 33-40] The present study is consistent with the overall recommendation from the aggregated investigations that UWS is preferred for allograft protection. The clinical trial data obtained with conventional organ ischemic times, that is, less than 4 hours, have been favorable[41-43] but cannot be considered conclusive.

The mechanism(s) responsible for the reported benefit of UWS with respect to cardiac storage is uncertain. The composition of UWS is unique, and several "UWS-like" formulations have been used experimentally. Ko and associates[44] have performed the most critical attempt to date to determine the importance of the different components of UWS using a servo-volume pump to evaluate left ventricular end-systolic and end-diastolic pressure-volume relationships. In their studies, an intracellular concentration of potassium, hydroxyethyl starch, and to a lesser degree lactobionate were considered to be essential factors. Adenosine, itself an important component, was included in all of the UWS preparations used in their studies.[3] Other investigations have suggested that further modification to the UWS formulation can provide additional benefits for cardiac allograft recovery.[6, 23, 45] Whether these findings can be translated into improvements in meaningful clinical end points is arguable. However, benefits would probably be observed after procurement of organs from high-risk donors[46] by virtue of prolonged anticipated organ ischemic time, excessive inotropic use, small size, or advanced age.

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